

CLAIMS

1. A method of analyzing an RNA sample comprising:
contacting an RNA sample with random primers under hybridization
conditions;

5 generating cDNA from the RNA sample by extending the random
primers with reverse transcriptase to produce cDNA;

degrading the RNA population;

fragmenting the cDNA;

labeling the cDNA fragments;

10 contacting the labeled cDNA fragments with a solid support
comprising nucleic acid probes under hybridization conditions; and

detecting the presence or absence of hybridization of the labeled
cDNA fragments to the nucleic acid probes on the solid support.

15 2. The method of claim 1 wherein, for the majority of RNAs in the
starting sample, the number of cDNA copies of a given sequence near the 3'
end of a single species of RNA is not more than twice the number of cDNA
copies of a given sequence near the 5' end of said single species of RNA.

20 3. The method of claim 1 wherein said RNA is selected from the group
consisting of total RNA, mRNA and poly(A)⁺ RNA.

4. The method of claim 1 wherein hybridization is detected by detecting
a signal from labeled DNA which is hybridized to the solid support.

25 5. The method of claim 1 wherein the cDNA fragments are labeled by
the addition of at least one labeled nucleotide using terminal transferase.

6. The method of claim 4 wherein the signal is amplified.

7. The method of claim 4 wherein the amount of signal detected with a probe to a 3' region of an RNA from the starting material is not more than twice the amount of signal detected with a probe to a 5' region of said RNA from the starting material.

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8. The method of claim 6 wherein the amount of signal detected with a probe to a 3' region of an RNA from the starting material is not more than twice the amount of signal detected with a probe to a 5' region of said RNA from the starting material.

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9. The method of claim 1 wherein the molar amount of cDNA fragments that hybridize to a probe to a 3' region of a RNA and the molar amount of cDNA fragments that hybridize to a probe to a 5' region of said RNA vary by 2 fold or less.

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10. The method of claim 1 wherein the solid support comprising nucleic acid probes is selected from the group consisting of a nucleic acid probe array, a membrane blot, a microwell, a bead, and a sample tube.

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11. The method of claim 1 wherein the random primers are 6 nucleotides in length.

12. The method of claim 1 wherein the random primers are 9 nucleotides in length.

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13. The method of claim 1 wherein the random primers are 15 nucleotides in length.

14. The method of claim 1 wherein the RNA sample is isolated from a prokaryotic cell.

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15. The method of claim 1 wherein the RNA sample is isolated from a eukaryotic cell or tissue.

5 16. The method of claim 15 wherein the eukaryotic cell or tissue is mammalian.

17. The method of claim 16 wherein the eukaryotic cell or tissue is human.

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18. The method of claim 1 wherein the RNA sample is isolated from a source selected from the group consisting of dissected tissue, microdissected tissue, a tissue subregion, a tissue biopsy sample, a cell sorted population, a cell culture, and a single cell.

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19. The method of claim 1 wherein the RNA sample is isolated from a cell or tissue source selected from the group consisting of brain, liver, heart, kidney, lung, retina, bone, lymph node, endocrine gland, reproductive organ, blood, nerve, vascular tissue, and olfactory epithelium.

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20. The method of claim 1 wherein the RNA sample is isolated from a cell or tissue source selected from the group consisting of embryonic and tumorigenic.

25 21. The method of claim 1 further comprising amplifying the cDNA fragments to produce amplified cDNA fragments.

22. The method of claim 21 further comprising:
contacting said amplified cDNA fragments with a solid support
30 comprising nucleic acid probes.

23. The method of claim 22 further comprising:
detecting the presence or absence of hybridization of said amplified
cDNA fragments to the nucleic acid probes on the solid support.

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24. The method of claim 23 wherein the solid support is selected from
the group consisting of a nucleic acid probe array, a membrane blot, a
microwell, a bead, and a sample tube.

10 25. The method of claim 1 wherein the RNA sample is further contacted
with primer comprising poly dT.

26. The method of claim 23 wherein hybridization is detected by
detecting a signal from labeled DNA which is hybridized to the solid support.

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27. The method of claim 26 wherein the signal is amplified.

28. A gene expression monitoring system comprising the labeled cDNA
fragments of Claim 1 and a solid support comprising nucleic acid probes.

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29. A gene expression monitoring system comprising the labeled cDNA
fragments of Claim 21 and a solid support comprising nucleic acid probes.

30. A kit for the detection of nucleic acids, wherein the kit comprises a
25 container, instructions for use, random primers, buffers, a reverse
transcriptase, DNase, a terminal transferase and an a solid support comprising
nucleic acid probes.

31. A method of detecting one or more isoforms of RNA in an RNA
30 sample comprising:

contacting an RNA sample with random primers under hybridization conditions;

generating cDNA from the RNA sample by extending the random primers with reverse transcriptase to produce cDNA;

5 degrading the RNA population;

fragmenting the cDNA;

labeling the cDNA fragments;

contacting the labeled cDNA fragments with an array comprising a probe that hybridizes to the complement of a sequence present in a first

10 isoform but absent from a second isoform; and

detecting the presence or absence of hybridization of the labeled cDNA fragments to the array.

32. The method of claim 31 wherein the RNA sample is selected from the group consisting of total RNA, poly(A)⁺ RNA and mRNA.

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33. The method of claim 31 wherein the RNA sample is isolated from a eukaryotic cell or tissue.

20 34. A method of detecting one or more isoforms of RNA in an RNA sample comprising:

contacting an RNA sample with random primers under hybridization conditions;

25 generating cDNA from the RNA sample by extending the random primers with reverse transcriptase to produce cDNA;

degrading the RNA population;

fragmenting the cDNA;

labeling the cDNA fragments;

contacting the labeled cDNA fragments with an array comprising a probe that hybridizes to the complement of a sequence common to each of the one or more isoforms; and

5 detecting the presence or absence of hybridization of the labeled cDNA fragments to the array.

35. The method of claim 34 wherein the RNA sample is selected from the group consisting of total RNA, poly(A)⁺ RNA and mRNA.

10 36. The method of claim 34 wherein the RNA sample is isolated from a eukaryotic cell or tissue.

37. A method of detecting all RNA transcripts of a single gene present in an RNA sample and distinguishing between different transcript isoforms
15 present in said RNA sample comprising:

contacting an RNA sample with random primers under hybridization conditions;

generating cDNA from the RNA sample by extending the random primers with reverse transcriptase to produce cDNA;

20 degrading the RNA population;

fragmenting the cDNA;

labeling the cDNA fragments;

contacting the labeled cDNA fragments with an array comprising a probe that hybridizes to the complement of a sequence present in each of the
25 one or more isoforms and a probe that hybridizes to the complement of a sequence common to each of the one or more isoforms; and

detecting the presence or absence of hybridization of the labeled cDNA fragments to the array.

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38. The method of claim 37 wherein the RNA sample is selected from the group consisting of total RNA, poly(A)⁺ RNA and mRNA.

39. The method of claim 37 wherein the RNA sample is isolated from a eukaryotic cell or tissue.

40. A method of detecting the presence or absence of transcriptional activity from a region of a genome comprising:

obtaining a sample of RNA transcribed from said genome;

10 contacting said RNA sample with random primers under hybridization conditions;

generating cDNA from the RNA sample by extending the random primers with reverse transcriptase;

degrading the RNA;

15 fragmenting the cDNA;

labeling the cDNA fragments;

contacting the labeled cDNA fragments with an array comprising probes that hybridize to a plurality of sequences present in said genome; and

20 detecting the presence or absence of hybridization of the labeled cDNA fragments to the array.

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